09/4191047

(FILE 'HOME' ENTERED AT 14:52:02 ON 01 APR 2002)

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 14:52:09 ON 01 APR 2002 32172 S LUCIFERASE LZ. 100 S L1 AND CASPASE L3 65 DUP REM L2 (35 DUPLICATES REMOVED) L4 . 35 S L3 AND CASPASE-3 4 S L4 AND RENILLA L5 FILE 'STNGUIDE' ENTERED AT 14:53:55 ON 01 APR 2002 FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 14:56:26 ON 01 APR 2002 L6 5 S L3 AND RENILLA L7 3 S L3 AND DETECT L8 42 S L3 AND ACTIVITY L9 6 S L8 AND PROTEASE L10 0 S RENILLA AND LUCIFERASE AND CONSERVED L11 0 S RENILLA AND LUCIFERASE AND CONSER? L12 0 S RENILLLA AND LUCIFERASE L13 369 S RENILLA AND LUCIFERASE L145 S L13 AND PROTEASE '5 DUP REM L14 (0 DUPLICATES REMOVED) L15 63 S L13 AND REGION L16 L17 33 DUP REM L16 (30 DUPLICATES REMOVED) L18 27 S L13 AND CLONING L19 25 DUP REM L18 (2 DUPLICATES REMOVED) L20 5 S L19 AND (SEA OR REINFORMIS) L21 1 S REINFORMIS AND LUCIFERASE L22 141 S RENIFORMIS AND LUCIFERASE L23 0 S L22 AND CASPASE

FILE 'STNGUIDE' ENTERED AT 15:08:43 ON 01 APR 2002

15 DUP REM L25 (1 DUPLICATE REMOVED)

0 S L22 AND CONSERVED

16 S L22 AND CLONING

L24

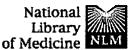
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ANSWER 10 OF 15 CAPLUS COPYRIGHT 2002 ACS
L26
     1998:221121 CAPLUS
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DN
     128:291113
     Renilla luciferase and green fluorescent protein fusion genes
TT
     Szalay, Aldar A.; Wang, Gefu; Wang, Yubao
IN
ΡÅ
     Loma Linda University, USA
SO
     PCT Int. Appl., 35 pp.
     CODEN: PIXXD2
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     English
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AB
     A fusion gene is provided comprising the cDNA of Renilla
     luciferase and the cDNA of the "humanized" Aequorea green
     fluorescent protein. The "RG fusion gene" was constructed with Renilla
     cDNA linked at a modified 3' end to a 15-nucleotide linker sequence
     encoding Ala-Ala-Ala-Ala-Thr, followed by the 5' end of intact GFP cDNA;
     similarly, a second "GR fusion gene" was constructed with GFP cDNA linked
     to a 27-residue linker sequence encoding Gly-Try-Gln-Ile-Glu-Phe-Ser-Leu-
     Lys, followed by the 5' end of Renilla cDNA. The RG fusion gene produces
     a novel protein, the "Renilla-GFP fusion protein", which displayed both
     the luciferase activity of Renilla luciferase, and the
     green fluorescence of GFP, whereas the GR fusion gene product exhibited
     minimal response to UV light and demonstrated no energy transfer between
     the GFP and Renilla luciferase moieties. The Renilla-GFP fusion
     gene is useful as a double marker for monitoring gene expression quant.
in
     UV light and by enzyme activity.
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☐ 1: Gene 1999 Sep 3;237(1):153-9

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ELSEVIER SCIENCE **FULL-TEXT ARTICLE**

Improved assay sensitivity of an engineered secreted Renilla luciferase.

Liu J, Escher A.

Center for Molecular Biology and Gene Therapy, Loma Linda University, CA, USA.

We have previously reported the construction of a functional Renilla luciferase enzyme secreted by mammalian cells when fused to the signal peptide of human interleukin-2. The presence of three predicted cysteine residues in the amino acid sequence of Renilla luciferase suggested that its secreted form could contain oxidized sulfhydryls, which might impair enzyme activity. In this work, four secreted Renilla luciferase mutants were constructed to investigate this possibility: three luciferase mutants in which a different cysteine residue was replaced by an alanine residue, and one luciferase mutant in which all three cysteine residues were replaced by alanine residues. Simian cells were transfected with the genes encoding these mutant luciferases, as well as with the original gene construct, and cell culture media were assayed for bioluminescence activity. Only media containing a mutated luciferase with a cysteine to alanine substitution at position 152 in the preprotein showed a marked increase in bioluminescence activity when compared to media containing the original secreted Renilla luciferase. This increase in light emission was due in part to enhanced stability of the mutant enzyme. This new enzyme represents a significant improvement in the sensitivity of the secreted Renilla luciferase assay for monitoring gene expression.

PMID: 10524246 [PubMed - indexed for MEDLINE]

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